Cytogenetic Studies in Laboratory Animals Exposed by Inhalation to Mainstream Smoke or Environmental Tobacco Smoke

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1 Introduction

Cytogenetic assays are an important means of assessing genotoxicity, both *in vitro* and *in vivo*. Sister chromatid exchange (Latt et al. 1983), chromosome aberration (Preston et al. 1983), and micronucleus (Heddle et al. 1983) assays have been used extensively as measures of cytogenetic damage in cultured cells, laboratory animals, and in humans. Cigarette smoke and its condensate have been widely evaluated for cytogenetic activity in *in vitro* experiments (DeMarini 1983; IARC 1986; Lee et al. 1987; Doolittle et al. 1990). While cigarette smoke and its condensate have been reported consistently to induce cytogenetic changes *in vitro*, studies on the potential of cigarette smoke to induce cytogenetic effects *in vivo* have not yielded uniform results. Lymphocytes from humans have been examined for cytogenetic damage in response to cigarette smoke, with inconsistent results. Some investigators have reported increases in chromosome aberrations in lymphocytes of smokers when compared to nonsmokers (Obe et al. 1978; Vijayalaxmi & Evans 1982; Obe et al. 1982) while others have reported no significant differences between smokers and nonsmokers (Bender et al. 1988; Bender et al. 1989). Some investigators have found increases in sister chromatid exchanges (SCE) of smokers (Carrano 1982; Lambert et al. 1978; Hopkin and Evans 1980; Reidy et al. 1988), and others have reported no significant differences (Hollander et al. 1978; Andrio et al. 1980; Crossen and Morgan 1980; Hedner et al. 1983). One laboratory reported significant increases in the frequencies of SCE in both lymphocytes and bone marrow cells of smokers (Kao-Shan et al. 1987). Inconsistent results also have been found when examining micronuclei in human lymphocytes. Some investigators have reported significant increases in micronucleus formation in lymphocytes of smokers (Lammendy and Knuutila 1991; Högstedt 1984; Högstedt et al. 1981; Högstedt et al. 1983; Izquierdo-Enguita and Simes-Porta 1989; Stenstrand 1985), while other

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investigators have not found significant increases (Au et al. 1991; Nordenson and Beckmann 1984; Obe et al. 1982). In summary, cytogenetic studies comparing human smokers and nonsmokers have yielded inconsistent results. It is probable that a host of factors, including occupation, medications, diet, age, and sex (Bender et al. 1988; Högstedt 1984; Wulf et al. 1986) influence cytogenetic results in humans, confounding efforts to study a specific agent like smoking. The use of an animal model would eliminate, or at least control for, the influence of many of these variables and would allow for mechanistic studies on the role of tobacco smoke or its specific components in the induction of cytogenetic effects. The objective of the present report was to evaluate the feasibility of using cytogenetic assays in bone marrow cells from rats or mice as indicators of genotoxicity following inhalation exposure to tobacco smoke. Data will be presented from two cytogenetic studies following inhalation exposure to mainstream cigarette smoke, namely a 90-day subchronic assay using Sprague-Dawley rats (Lee et al. 1990; Coggins et al. 1989) and a 14-day inhalation study using B6C3F1 mice (Coggins et al. 1990). Sister chromatid exchange (SCE), chromosome aberration, and micronuclei (MN) in bone marrow cells were evaluated. Bone marrow cells were chosen since these cells were used in previous animal inhalation studies with tobacco smoke (Putman et al. 1985; Benedict et al. 1984 Balansky et al. 1987; Korte et al. 1981).

Recently, concern about the biological significance of exposure to environmental tobacco smoke (ETS) has emerged as a subject of intense public discussion. Thus, additional studies were conducted to determine if exposure to ETS alters chromosome aberration frequencies in pulmonary alveolar macrophages (PAM) in rats exposed subchronically to ETS.

All studies used the 1R4F reference cigarette (Davis et al. 1984), obtained from the Tobacco and Health Research Institute (Lexington KY). The 1R4F is a filtered cigarette, with a yield of approximately 10 mg total particulate matter (TPM) per cigarette, designed to reflect the average composition of cigarettes in the U.S. market.

2 90-Day subchronic inhalation study in rats exposed to mainstream cigarette smoke

Male and female Sprague-Dawley rats were exposed nose-only to mainstream smoke 1 h per day, 5 days per week for 13 consecutive weeks (Coggins et al. 1989; Lee et al. 1990). Animals were exposed to 200 or 400 mg TPM/m³. Four males and four females were taken from groups of exposed animals and their bone marrow examined. In addition, 3 male and 3 female rats were injected i.p. with either cyclophosphamide (10 mg/kg b.w.) or Mitomycin C (2 mg/kg b.w.) as positive controls, and with phosphate-buffered saline (PBS, 1 ml/kg b.w.) as vehicle controls at the end of the 90-day test period. Sister chromatid exchange, chromosome aberration, and micronucleus assays were conducted as described earlier (Lee et al. 1990). Rats in the high exposure group had 22 % COHb and 90.1 ng of nicotine/ml plasma. Histopathological changes were noted in the smoke-exposed groups (Coggins et al. 1989): hyperplasia, as well as squamous metaplasia, in nasal 1; chronic active inflammation and squamous metaplasia in the ventral larynx, and accumulation of nonpigmented and brown-gold macrophages in the lungs. These parameters all indicated inhalation of large quantities of the smoke presented to the animals.

2.1 Chromosome aberration

Results from chromosome aberration assays are presented in Table 1. The percentage of cells with aberrations in the smoke-exposed groups were not significantly different than those of the negative control groups. Aberrations observed in both exposed and negative control groups consisted mainly of chromatid breaks and isochromatid breaks. Animals treated with Mitomycin C showed elevated aberration frequencies in both male and female rats.

Table 1: Chromosome Aberrations in Bone-Marrow Cells of Sprague-Dawley Rats Exposed to Mainstream Smoke from 1R4F Reference Cigarettes for 90 days[a]

Exposure Group	Numbers of Aberrations per Cell	% Cells with Aberrations[c]
Males		
Room Control	0.050	0.5
Vehicle Control[b]	0.020	1.3
Positive Control[c]	0.190	15.3[d]
Ref. Cigarette[f]		
200 mg/m³	0.025	2.5
400 mg/m³	0.035	3.0
Females		
Room Control	0.050	0.5
Vehicle Control[b]	0.027	2.7
Positive Control[c]	0.290	21.3[d]
Ref. Cigarette[f]		
200 mg/m³	0.010	1.0
400 mg/m³	0.010	1.0

[a] Lee et al. (1990)
[b] Vehicle Control: physiological saline at 1 ml/kg body weight.
[c] Positive control: Mitomycin C at 2 mg/kg body weight.
[d] Significantly greater than control by Fisher's Exact test ($p < 0.05$).
[e] 50 metaphase cells/animal were scored for aberrations.
[f] Animals exposed 1 h per day, 5 days per week, 13 consecutive weeks. Rats were killed 9 h after the last inhalation exposure.

2.2 SCE assay

Sister chromatid exchange results are reported in Table 2. Cell-cycle kinetic comparisons revealed no significant difference between rats in the negative control and the smoke-exposed groups, indicating that none of the smoke exposures caused cell cycle delay in the bone marrow. The SCE frequencies in the smoke-exposed groups were within the range of the negative control groups. Animals treated with the positive control, however, had significantly elevated SCE frequencies ($p < 0.05$).

Table 1: Sister Chromatid Exchanges in Bone-Marrow Cells of Sprague-Dawley Rats Exposed to Mainstream Smoke from 1R4F Reference Cigarettes for 90 days[a]

Exposure Group	M1	% Cell M1+	Cycle M2	Stage M2+	# of SCE/Cell[e] ±S.D.
Males					
Room Control	41	1	52	6	6.13 ± 2.06
Vehicle Control[b]	11	0	85	4	7.11 ± 2.12
Positive Control[c]	48	3	49	0	37.47 ± 8.95[d]
Ref. Cigarette[f]					
200 mg/m³	48	0	50	2	6.96 ± 2.30
400 mg/m³	52	0	44	4	6.18 ± 1.55
Females					
Room Control	23	4	69	4	6.33 ± 2.00
Vehicle Control[b]	24	0	72	4	7.10 ± 1.62
Positive Control[c]	52	5	43	0	33.96 ±13.19[d]
Ref. Cigarette[f]					
200 mg/m³	39	7	50	4	6.44 ± 2.50
400 mg/m³	43	0	57	0	6.72 ± 1.66

[a]Lee et al. (1990)
[b]Vehicle Control: physiological saline at 1 ml/kg body weight.
[c]Positive Control: cyclophosphamide at 10 mg/kg body weight.
[d]Significantly different from vehicle controls at $p < 0.05$.
[e]50 M2 cells/animal were scored for SCE.
[f]Animals exposed 1 h per day, 5 days per week 13 consecutive weeks

2.3 Micronucleus assay

The results of the micronucleus assay in bone marrow cells are shown in Table 3. Cytotoxicity, indicated by PCE:NCE (polychromatic erythroblasts:normochromatic erythroblasts) ratios, was not different in smoke-exposed groups compared to negative control groups. No significant differences were observed in the frequencies of micronucleated PCE (MN-PCE) between smoke-exposed groups and negative control groups. Animals treated with the positive control cyclophosphamide showed a statistically significant increase ($p \sim 0.0227$) for both males and females in the frequency of MN-PCE over the negative control animals.

Table 3: Micronucleus Assay in Bone-Marrow Cells of Sprague-Dawley Rats Exposed to Mainstream Smoke from 1R4F Reference Cigarettes for 90 days[a]

Exposure Group	PCE:NCE Mean ± SD	%MN-PCE[e] Mean ± SD
Males		
Room Control	0.438 ± 0.132	0.75 ± 0.25
Vehicle Control[b]	0.461 ± 0.232	0.63 ± 0.06
Positive Control[c]	0.328 ± 0.049	2.10 ± 0.66[d]
Ref. Cigarette[f]		
200 mg/m³	0.544 ± 0.218	0.68 ± 0.31
400 mg/m³	0.427 ± 0.106	0.48 ± 0.28
Females		
Room Control	0.466 ± 0.108	0.45 ± 0.20
Vehicle Control[b]	0.640 ± 0.046	0.63 ± 0.06
Positive Control[c]	0.365 ± 0.036	0.97 ± 0.12[d]
Ref. Cigarette[f]		
200 mg/m³	0.473 ± 0.225	0.60 ± 0.32
400 mg/m³	0.451 ± 0.206	0.38 ± 0.21

[a]Lee et al. (1990)
[b]Vehicle control: physiological saline at 1 ml/kg body weight.
[c]Positive Control: cyclophosphamide at 10 mg/kg body weight.
[d]Significantly different than vehicle controls by the Mann-Whitney test at $p < 0.05$.
[e]1000 PCE/Animal were scored.
[f]Animals exposed 1 h per day, 5 days per week, 13 consecutive weeks.

In conclusion, rats subchronically exposed to very high doses of mainstream cigarette smoke, as indicated by high COHb, high plasma nicotine levels, and histopathological changes in the respiratory tract, did not exhibit cytogenetic effects in bone marrow as measured by SCE, MN, and chromosome aberration assays.

3 14-Day inhalation study in mice exposed to mainstream cigarette smoke

Male and female B6C3F1 mice were exposed nose-only to three concentrations of mainstream smoke from 1R4F Reference cigarettes for 14 consecutive days, 1 h per day (Coggins et al. 1990). Smoke concentrations were adjusted to 0, 160, 320, and 640 mg TPM/m³. Four animals per sex per exposure group were used for SCE, MN, and chromosome aberration assays. Sister chromatid exchange, chromosome aberration, and micronucleus assays were conducted as described previously (Lee 1990). Blood COHb concentrations were 14.9 % ± 1.4 (S.D.), 24.6 % ± 3.1, and 38.8 % ± 3.0 for low, medium, and high exposures. Plasma nicotine values were 79.5, 112, and 138 ng/ml, respectively. Negligible values for COHb and plasma nicotine were obtained from sham-exposed animals. These markers of dosimetry confirm that large amounts of cigarette smoke were inhaled by animals in the smoke-exposed groups. This was further evidenced by the presence of histopathological changes in smoke-exposed animals (Coggins et al. 1990): mild epithelial hyperplasia in nasal I (medium and high groups only, both sexes), mild to moderate focal epithelial hyperplasia in the ventral larynx (all groups, both sexes), mild to moderate focal squamous metaplasia of the ventral laryngeal epithelium (all groups, both sexes) and pulmonary brown-gold macrophages in the lung.

3.1 Chromosome aberration assays

There were no significant increases in the frequencies of cells with chromosome aberrations in any of the smoke-exposed groups (Table 4). Aberrations observed in both exposed and negative control groups were primarily chromatid and isochromatid breaks. Animals treated with the positive control cyclophosphamide showed significant increases in per cent cells with aberrations.

Table 4. Results of Chromosome Aberration in Bone-marrow Cells of B6C3F1 Mice Exposed to Mainstream Smoke from 1R4F Reference Cigarettes for 14 Consecutive Days (1 h/day)[a]

Exposure Group[b]	Numbers of Aberrations per Cell	% Cells with Aberrations[c]
Males		
Sham	0.00	0.0
Room	0.01	1.0
Positive control[d]	0.45	20.0[e]
Reference Cigarette		
160 mg/m³	0.01	1.5
320 mg/m³	0.02	1.5
640 mg/m³	0.00	0.0
Females		
Sham	0.01	1.0
Room	0.01	1.0
Positive control[d]	0.15	13.0[e]
Reference Cigarette		
160 mg/m³	0.04	3.0
360 mg/m³	0.03	2.5
640 mg/m³	0.00	0.0

[a] Coggins et al. (1990)
[b] Four animals per group.
[c] Cyclophosphamide, 25 mg/kg
[d] Significantly greater than controls by Fisher's exact test ($p < 0.05$).
[e] 50 metaphase cells/animal (200 per exposure group) were scored for aberrations.

3.2 Sister Chromatid Exchange

The results of the SCE assays are presented in Table 5. Cell cycle kinetics showed no significant differences between the sham-exposed, room control, and smoke-exposed groups. SCE frequencies in the smoke-exposed groups were not statistically different (ANOVA) from those in sham-exposed animals. The positive control, cyclophosphamide, induced a significant increase in SCE frequency in both sexes.

Table 5. Results of Sister Chromatid Exchange in Bone-marrow Cells of B6C3F1 Mice Exposed to Mainstream Smoke from 1R4F Reference Cigarettes for 14 Consecutive Days (1 h/day)[a]

Exposure Group[b]	% Cell Cycle Stage					# SCE/Cell +/-S.D.
	M1	M1+	M2	M2+	M3	
Males						
Sham	13	6	62	1	18	5.36 ± 0.46
Room	5	11	65	4	15	5.47 ± 1.07
Positive control[c]	12	20	50	10	8	17.78 ± 2.47[d]
Reference Cigarette						
160 mg/m³	9	9	53	11	18	6.25 ± 0.28
320 mg/m³	12	1	73	0	14	5.04 ± 0.28
640 mg/m³	11	7	60	5	17	6.64 ± 1.53
Females						
Sham	9	6	63	0	22	5.53 ± 0.44
Room	9	3	67	3	18	5.17 ± 0.48
Positive control[c]	14	6	66	0	14	15.29 ± 4.25[d]
Reference Cigarette						
160 mg/m³	28	9	35	0	8	6.86 ± 1.00
340 mg/m³	8	3	67	1	21	6.27 ± 1.06
640 mg/m³	16	0	39	0	23	5.52 ± 0.48

[a]Coggins et al. (1990).
[b]Four animals per group.
[c]Cyclophosphamide, 25 mg/kg.
[d]Significantly greater than sham controls at $p < 0.05$.

3.3 Micronucleus Assay

Results from the micronucleus assay are presented in Table 6. Cytotoxicity, indicated by PCE:NCE ratios, was not different in smoke-exposed groups compared to negative control groups. There was a minimal increase in MN frequency at the highest dose only. Statistical analyses indicate that this slight increase may be interpreted as either significant or not, depending on the statistical test applied. Analysis of variance indicated no significant difference among sham controls and smoke-exposed animals. However, linear trend tests showed significant increases ($p < 0.05$) in reference groups, both male and female.

Table 6: Micronucleus Assay in Bone-Marrow Cells of B6C3F1 Mice Exposed to Mainstream Smoke from 1R4F Reference Cigarettes for 14 Consecutive Days (1 h/day)[a]

Exposure Group[b]	PCE:NCE (± SD)	% MN-PCE (± SD)
Males		
Sham Control	0.603 ± 0.172	0.38 ± 0.05
Room Control	0.827 ± 0.130	0.33 ± 0.10
Positive Control[c]	0.878 ± 0.225	1.38 ± 0.26[d]
Positive Control[e]	0.871 ± 0.128	2.00 ± 0.34[d]
Ref. Cigarette		
160 mg/m³	0.480 ± 0.112	0.40 ± 0.28
320 mg/m³	0.719 ± 0.295	0.40 ± 0.14
640 mg/m³	0.797 ± 0.058	0.60 ± 0.08[d]
Females		
Sham Control	0.599 ± 0.262	0.35 ± 0.13
Room Control	0.594 ± 0.133	0.28 ± 0.17
Positive Control[c]	0.824 ± 0.196	1.55 ± 0.46[d]
Positive Control[e]	0.699 ± 0.219	1.30 ± 0.35[d]
Ref. Cigarette		
160 mg/m³	0.737 ± 0.203	0.35 ± 0.34
320 mg/m³	0.925 ± 0.093	0.45 ± 0.33
640 mg/m³	0.841 ± 0.116	0.70 ± 0.12[d]

[a]Coggins et al. (1990).
[b]Four animals per group.
[c]Positive control: cyclophosphamide at 12.5 mg/kg, 48 h after injection.
[d]Significantly different than vehicle controls by the Mann-Whitney U test at $p < 0.05$.
[e]Cyclophosphamide at 12.5 mg/kg, 24 h after injection.

In conclusion, while histopathologic changes were observed in mice exposed to large amounts of mainstream smoke (indicated by COHb and plasma nicotine levels), negative results were obtained in SCE and chromosome aberration assays, with only a marginal increase in micronucleus frequency.

4 The assessment of chromosome aberrations in pulmonary alveolar macrophages (PAMs) of rats exposed to mainstream tobacco smoke

Shortly after completion of our studies on bone marrow cells of tobacco smoke-exposed rodents, Rithidech et al. (1989) reported a significant increase in chromosome aberrations in pulmonary alveolar macrophages of mainstream smoke-exposed rats (Fischer 344/N). In this study, rats were exposed to approximately 100 to 200 mg TPM/m³ for 6 h/day, 5 days/week for 22 to 24 days. Cigarettes were unfiltered, high-tar (27 mg TPM), high nicotine (1.5 mg) 1R3 research cigarettes (Tobacco Health Research Institute, Lexington, KY). Results of the chromosome aberration assays are reported in Table 7.

Table 7: Chromosome Aberrations in Pulmonary Alveolar Macrophages from Rats Exposed to Mainstream Cigarette Smoke[a,b]

Exposure Route	Sham Control	Cigarette Smoke Exposed[c]
Whole Body	1.0 ± 0.58	8.50 ± 2.20[d]
Nose Only	3.0 ± 1.91	12.00 ± 2.58[d]

[a]Rithidech et al. (1989).
[b]Percentage of cells with aberrations, mean ±S.E.
[c]200 mg/m³, 6 h per day 22-24 days.
[d]Significantly elevated relative to the matched control groups.

Animals exposed either nose-only or whole-body exhibited significantly elevated numbers of PAMs with chromosome aberrations. Rithidech et al. (1989) speculated that the aberrations detected in this study may have resulted from damage accumulated over the cell cycle prior to harvest since PAMs are a transient population in the lung, with only a small fraction dividing, and a short cell cycle (9-10 h).

5 The assessment of chromosome aberrations in PAMS of rats exposed to Environmental Tobacco Smoke

The potential health consequences of exposure to environmental tobacco smoke have been widely discussed by scientists and the general public (Department of Health and Human Services 1986; National Research Council 1986). Detection of chromosome aberrations in PAMs of tobacco smoke-exposed rats (Rithidech et al. 1989) indicated that PAMs may be more sensitive target cells for cytogenetic changes than bone marrow cells following inhalation of mainstream tobacco smoke. This method of evaluating chromosome aberrations was therefore included along with the measurement of DNA adducts in lung tissue when studies were conducted to determine if exposure to environmental tobacco smoke (ETS) induces genotoxicity in the lungs of rats (Lee et al. 1992; Lee et al. 1993). Sprague-Dawley rats were exposed nose-only to 0, 0.1, 1.0, and 10 mg TPM/m³ of aged and diluted sidestream smoke (ADSS), a surrogate for environmental tobacco smoke, for 6 h per day (Lee et al., 1992; Coggins et al. 1992; Lee 1993; Coggins et al. 1993). The lowest concentration, 0.1 mg TPM/m³ represents a typical concentration of human ETS exposure in rooms where smoking is unrestricted (Guerin et al. 1992). Alveolar macrophages from animals exposed for 7, 28, and 90 days were examined for chromosome aberrations. The results are presented in Table 8.

Although chromosome aberrations were not found in PAMs, DNA adducts were detected in lung tissue of animals exposed to the 10 mg TPM/m³ dose for 7, 28, and 90 days. Analyses were conducted using the ^{32}P-postlabeling assay (Randerath et al. 1981; Reddy and Randerath 1986, Gupta et al. 1982), with the nuclease P1 procedure (Reddy and Randerath 1986). Maps of lung DNA adducts from animals in the 10 mg TPM/m³ exposure group exhibited slight diffuse diagonal radioactive zones (DRZ) after 7, 28, and 90 days of exposure. Animals in the 0.1 mg/m³, the 1.0 mg/m³ and the sham-exposed groups did not have DRZ at any time point.

Table 8: Chromosome Aberration Analysis in Pulmonary Alveolar Macrophages of Rats Exposed to Aged and Diluted Sidestream Smoke[a]

Exposure Group	% Cells with Aberration
7 DAYS	
Sham (Room air)	1.3
Low (0.1 mg/m³)	1.1
Medium (1.0 mg/m³)	0
High (10.0 mg/m³)	4.0
28 DAYS	
Sham (Room air)	3.2
Low (0.1 mg/m³)	2.8
Medium (1.0 mg/m³)	2.0
High (10 mg/m³)	4.0
90 DAYS	
Sham (Room air)	1.2
Low (0.1 mg/m³)	0.6
Medium (1.0 mg/m³)	0.8
High (10 mg/m³)	1.8
Cyclophosphamide (10 mg/kg)	13.5[b]

[a] Lee et al., (1993, 1993).
[b] Significantly different than sham control ($p < 0.05$)

6 Evaluation of cytogenetic assays in tobacco smoke-exposed animals

In-vivo bone-marrow cytogenetic studies with rodents exposed to cigarette smoke have produced mixed results. No positive SCE or chromosome aberration results in bone marrow cells of rats (Lee et al. 1990) or hamsters (Korte et al. 1981) have been reported. Mice are the only rodent species reported to have cytogenetic alterations, and then only after exposure to high doses of high-tar

cigarettes. Slight increases in the frequencies of SCE (Benedict et al. 1984; Putnam et al. 1985) and in the micronucleus assay (Balansky et al. 1987, 1988; Coggins et al. 1990) have been reported in mice following exposure to cigarette smoke. Benedict et al. (1984) used unfiltered cigarettes with published yields of 38-45 mg TPM in intermittent exposures at extremely high smoke concentrations (9000 mg TPM/m³). Putnam et al. (1985) reported increases in SCE at similarly high concentrations (4000 and 9000 mg TPM/m³) of smoke from high tar unfiltered cigarettes. Balansky et al. (1987 and 1988) reported an increase in micronucleated PCE at an unspecified smoke concentration in BDF1 mice, and Coggins et al. (1990) saw marginal increases in MN only at a very high smoke concentration.

Little information has been published to date on cytogenetic assays in circulating lymphocytes in rodents following exposure to mainstream tobacco smoke. Basler et al. (1982) exposed Wister rats to smoke from unfiltered cigarettes, and found no significant increase in SCE in lymphocytes. Balansky et al. (1988) reported increases in micronucleated NCE in BDF1 mice exposed to an unknown concentration of smoke. Significant increases in chromosome aberrations were reported in PAMs of rats exposed to tobacco smoke (Rithidech et al. 1989).

More data are needed to determine an appropriate animal model for the assessment of cytogenetic effects of tobacco smoke. Based on this laboratory's experience and on published reports, bone marrow cytogenetic analysis is not sufficiently sensitive to evaluate the genotoxic potential of tobacco smoke in laboratory animals. This may be due in part to the rapid rate at which bone marrow proliferates, precluding the accumulation of cytogenetic damage from chronic or subchronic exposure. Long-lived circulating lymphocytes may be a more appropriate target cell than are bone marrow cells since lymphocytes may accumulate cytogenetic damage over a long period of time.

The recently developed technique of fluorescence *in situ* hybridization with chromosome-specific DNA probes ("chromosome painting") (Lucas et al. 1989, Pinkel et al. 1988, Tucker et al. 1992; respective articles in this volume) may prove to be a useful cytogenetic tool in studying the effects of smoking on lymphocytes and other cells in humans. This technique is a relatively rapid method for detecting stable chromosome translocations. Similar techniques are being developed for use in mice (Natarajan et al. 1992). Further studies should focus on a comparison of cytogenetic damage in bone marrow cells, circulating lymphocytes, and pulmonary alveolar macrophages in order to investigate the relative sensitivity, advantages, and limitations of each system.

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